U.S. PATENT APPLICATION

ENTITLED:

METHOD, ARRANGEMENT, AND SOFTWARE FOR OPTIMIZING THE IMAGE QUALITY OF MOVABLE SUBJECTS IMAGED WITH A MICROSCOPE

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METHOD, ARRANGEMENT, AND SOFTWARE FOR OPTIMIZING THE IMAGE QUALITY OF MOVABLE SUBJECTS IMAGED WITH A MICROSCOPE

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority of the German patent application 102 35 657.2 which is incorporated by reference herein.

FIELD OF THE INVENTION

The invention concerns a method for optimizing the image quality of image sequences of movable subjects acquired with a microscope.

The invention further concerns an arrangement for optimizing the image quality of image sequences of movable subjects acquired with a microscope.

BACKGROUND OF THE INVENTION

In the observation of living and movable subjects, artifacts occur in image production because the subjects move. This on the one hand results in unsharpness (motion generates artifacts similar to those of defocusing), and on the other hand, in confocal microscopy the images exhibit poor quality (signal-to-noise ratio) because methods such as image averaging cannot be applied to a pixel when motion is present. With averaging, for example, motion would cause subject pixels to be mixed with non-subject pixels.

SUMMARY OF THE INVENTION

It is the object of the invention to create a method with which it is possible to generate high-quality images of movable subjects, and to make

possible efficient application of operations such as averaging and filtering, even to movable subjects.

The object is achieved by means of a method for optimizing the image quality of movable subjects imaged with a microscope system, comprising the following steps:

a) acquiring a plurality of images having a plurality of pixels;
b) determining a respective displacement vector field from a comparison of the pixels of each two chronologically successive images;
c) identifying a trajectory for each pixel of the image from the displacement vector fields; and
d) applying an operation to the image data along a trajectory.

A further object of the invention is to create an arrangement with which it is possible to generate high-quality images of movable subjects, and to make possible efficient application of operations such as averaging and filtering, even to movable subjects.

The object is achieved by way of an arrangement for optimizing the image quality of movable subjects imaged with a microscope system, the microscope system comprising: at least one objective defining an image window, a detector unit for acquiring a plurality of images each having a plurality of pixels, a computer system, which encompasses a means for determining a respective displacement vector field from a comparison of the respective pixels of at least two chronologically successive images, a means for identifying a trajectory for each pixel of the image from the displacement vector fields, and a means for applying an operation to the image data along a trajectory.

In order to solve the problem associated with these objects, it is advantageous that a trajectory, which records displacements and thus subject motions, is determined for each pixel of the image. The displacements and subject motions are advantageously determined as displacement vector fields which evaluate in their totality all of the motions within the scene. The displacement vector field results

from a comparison of the pixels of, in each case, at least two chronologically successive images. The use of more than two images of a sequence may result in better convergence. Such displacement fields are determined by solving a flow problem, a pixel change model being formulated as a differential equation and fitted numerically to the image data using a minimum description length (MDL) method. Probably the most prominent representative of such models is the modeling of the motion of solid bodies in video technology, for which the synonym "optical flow method" has already become established. Further representatives may be found, for example, in climate modeling, where liquid bodies (from clouds to water) are modeled. Although the "optical flow" designation is not common here, this text uses the term synonymously. A trajectory is constructed by tracking the local displacement vectors from pixel to pixel, which can easily be accomplished with a computer algorithm. The trajectory determined in this fashion is a so-called guideline for the application of operations. Operations along the trajectory that is determined can be, for example (with no limitation as to generality), a deconvolution, a smoothing, or an averaging filter. An extension to the entire class of image processing classes operating in time-lateral fashion is included in this application in this context, and is left to the imagination of one skilled in the art in terms of implementing a system.

A peculiarity of these new operations is that ambiguities occur as a result of the motion and the displacement vector field. For example, a subject can migrate into the image segment, and a filter with memory must treat that new pixel differently from another, more-static pixel in the same scene. Another example is the splitting of a subject into several subjects (trajectory source). Yet another is the combination of individual pixels into one (trajectory sink). This is solved by way of intelligent trajectory management.

Further advantageous embodiments of the invention are evident from the dependent claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The subject matter of the invention is depicted schematically in the drawings and will be explained below with reference to the Figures, in which:

FIG. 1 schematically depicts a scanning microscope;

FIG. 2 schematically depicts the image frame imaged through the microscope, and the manner in which it is subdivided into individual regions or pixels;

FIG. 3 schematically depicts the processing of the data obtained from the observation of living and movable subjects;

FIG. 4 is a block diagram of the method according to the present invention;

FIG. 5 depicts an example of a situation in which a subject leaves the image, and the identified trajectory ends at the edge;

FIG. 6 depicts an example of a situation in which a subject comes into the image;

FIG. 7 depicts a situation in which a subject splits and several trajectories result therefrom; and

FIG. 8 depicts a situation in which several subjects combine into one subject, and the trajectories of the individual subjects end at one point.

DETAILED DESCRIPTION OF THE INVENTION

FIG. 1 schematically shows an exemplary embodiment of a confocal scanning microscope system with which the method according to the present invention can be carried out. Other microscope systems can likewise be used. A detector unit 19 is equipped with a video system or a CCD sensor for acquiring images.

This is not, however to be construed as a limitation of the invention. It is sufficiently clear to one skilled in the art that the invention can also be carried out with conventional microscopes with digital image production. Illuminating light beam 3 coming from at least one illumination system 1 is directed by a beam splitter or a suitable deflection means 5 to a scanning module 7. Before illuminating light beam 3 strikes deflection means 5, it passes through an illumination pinhole 6. Scanning module 7 comprises a gimbal-mounted scanning mirror 9 that guides illuminating light beam 3 through a scanning optical system 12 and a microscope objective 13, over or through a subject 15. In the case of non-transparent subjects 15, illuminating light beam 3 is guided over the subject surface. With biological subjects 15 (preparations) or transparent subjects, illuminating light beam 3 can also be guided through subject 15. For that purpose, non-luminous preparations are optionally prepared with a suitable dye (not depicted, since established existing art). The dyes present in the subject are excited by illuminating light beam 3 and emit light in a characteristic spectral region peculiar to them. This light proceeding from subject 15 defines a detected light beam 17. The latter travels through microscope optical system 13 and scanning optical system 12 and via scanning module 7 to deflection means 5, passes through the latter and arrives, through a detection pinhole 18, at at least one detector unit 19, which is equipped in the exemplary embodiment depicted here with at least one photomultiplier as detector. It is clear to one skilled in the art that other detectors, for example diodes, diode arrays, photomultiplier arrays, CCD chips, or CMOS image sensors, can also be used. Detected light beam 17 proceeding from or defined by subject 15 is depicted in FIG. 1 as a dashed line. In detector 19, electrical detected signals proportional to the power level of the light proceeding from subject 15 are generated. Since, as already mentioned above, light of more than one wavelength is emitted from subject 15, it is useful to insert in front of detector unit 19 a selection means 21 for the spectrum proceeding from the sample. The data generated by detector unit 19 are forwarded to a computer system 23. At least one peripheral unit 27 is associated with computer system 23. The peripheral unit can be, for example, a display on which the user receives instructions for adjusting the scanning microscope and can also view the present

setup and also the image data in graphical form. Also associated with computer system 23 is an input means comprising, for example, a keyboard 28, an adjusting apparatus 29 for the components of the microscope system, and a mouse 30.

FIG. 2 schematically depicts an image frame 41 acquired with microscope 100. Image frame 41 is defined by the image window determined by microscope 100. Image frame 41 is subdivided into individual regions or pixels 39. Movable subject 40 is located within image frame 41. Pixels 39 can be embodied as two-dimensional regions of image frame 41, or also as three-dimensional regions of image frame 41.

FIG. 3 shows the observation of living and movable subjects 40 and the processing of data obtained from the observation of living and movable subjects 40. For the observation of living and movable subjects 40, several images or image frames 41_1, 41_2, 41_3, ..., 41_n are acquired consecutively, for example using scanning microscope 100 described in FIG. 1, each image frame 41_1, 41_2, 41_3, ..., 41_n defining an XY plane or an acquired specimen volume XYZ. Between each two successive images, e.g. 41_1, 41_2 or 41_2, 41_3, or 41_{n-1}, 41_n, a respective displacement vector field 42_1, 42_2, ..., 42_{n-1} is determined. The displacement vector field between two successive images, e.g. 41_2 and 41_3, can be determined from a comparison of the individual mutually corresponding pixels of the two images. Proceeding from a first image 41_1 having N pixels, it is thus possible to ascertain the new positions in the next image 42_2 by way of the displacement. An even more accurate model can also be fitted for a trajectory 43, with sub-pixel accuracy, from the discrete displacements. Advantageously, more than one successive image is then used for this accuracy-enhancing operation. Trajectory 43 for the movable subject is obtained from the plurality of displacement vector fields 42_1, 42_2, ..., 42_{n-1} by tracking the displacement vector fields of the individual images 41_1, 41_2, 41_3, ..., 41_n. In the graphical depiction of trajectory 43, the moving subjects are represented by at least one trajectory through XYt space 44.

A video contains a three-dimensional space-time (two spatial dimensions XY, one time dimension t). The pixels of a movable subject 40 thus move along a curved path (trajectory) within this space-time. Trajectory 43 that is determined defines this curved path unequivocally, and data concerning the motion of subject 40 are thereby obtained. Operations that are to be applied to the moving subject can thus be performed along trajectory 43. For example, data about said trajectory 43 can be fed to an averaging filter, yielding an image of higher quality that takes into account the motion of subject 40, specifically in that the signal-to-noise ratio is better. This approach is of course also possible for sequences of volumes (four-dimensional space-time), and can be transferred to any kind of operation, e.g. filters (deconvolution, smoothing). In order to produce these filters, instead of the simple summing formulas common in image processing, the continuous operation equation must be discretized to the trajectory in the curved space-time, incorporating the present geometry. Such methods are established in numerical mathematics, and are existing art in simulation technology.

FIG. 4 is a block diagram of the method according to the present invention. The first step is image acquisition 50 of a series of images. As already described above, acquisition is accomplished using detector unit 19 of the microscope or scanning microscope. The data representing each image are stored in a first image memory 52. From image memory 52, the images are sequentially conveyed to an optical flow calculator 53. Parallel therewith, the data of each image are conveyed to a nonlinear filter 54. From optical flow calculator 53, the data modified by calculator 53 are conveyed to a trajectory tracker 55 and then to a trajectory memory 56. The data present in trajectory memory 56 are also made available to nonlinear filter 54 in order to allow discretization. As already mentioned above, any desired operations are applied to the acquired image data, taking place in nonlinear filter 54 in consideration of the stored trajectory 43. The data modified in this fashion travel into a second image memory 58 and can be retrieved from there, for example, for presentation on a display.

FIGS. 5 through 8 depict various events that result in respectively distinguished trajectories. FIG. 5 depicts the situation in which subject 40 leaves image frame

41 during the recording of N image frames. Upon recording of the (N+1)th image frame, the subject can no longer be captured by the microscope. Trajectory 43 resulting from the N captured image frames ends at the edge of XYt space 44. It can be deleted from trajectory memory 56 by trajectory tracker 55.

FIG. 6 depicts the situation in which a subject 40 is present in first image frame 41_1. During the recording of N image frames, a further subject 60 enters the region of the image frame, so that it can be captured by the microscope. Subject 60 can also be captured by the microscope when the Nth image frame is recorded. In addition to trajectory 43 for subject 40, a further trajectory 63 is added in XYt space 44 for subject 60 that has just entered the image frame of the microscope.

FIG. 7 depicts the situation in which a subject 70 is present in first image frame 41_1. By the time the Nth image frame is recorded, subject 70 has split into, for example, four subjects 70_1, 70_2, 70_3, and 70_4. Subjects 70_1, 70_2, 70_3, and 70_4 can also be captured by the microscope upon recording of the Nth image frame. In addition to trajectory 43 for subject 70, four further trajectories 73_1, 73_2, 73_3, and 73_4 are added at an end point of trajectory 43 at a certain time t, representing the motions of four subjects 70_1, 70_2, 70_3, and 70_4.

FIG. 8 depicts the situation in which four subjects 80_1, 80_2, 80_3, and 80_4 are present in first image frame 41_1. Upon recording of the Nth image frame, subjects 80_1, 80_2, 80_3, and 80_4 have combined into one subject 80. Trajectories 83_1, 83_2, 83_3, and 83_4 end in XYt space 44 at a point 84.

The invention has been described with reference to a particular exemplary embodiment. It is self-evident, however, that changes and modifications can be made without thereby leaving the range of protection of the claims below.